

**In the Specification:**

Please replace the paragraph beginning at page 3, line 19, with the following:

--**Figure 3** is a chart which illustrates one advantage achieved through the use of PPPA and PPG in MGB-modified oligonucleotide probes. As seen in the figure, the modified bases allow shortening of the probe that shows increased mismatch discrimination in real-time PCR. Ñ is PPPA and Œ is PPG. MGB-modified FAM probe = SEQ ID NO:1; MGB-modified FAM probe complement = SEQ ID NO:2; PPPA and PPG containing MGB-modified FAM probe complement = SEQ ID NO:3; PPPA and PPG containing MGB-modified FAM probe = SEQ ID NO:4.--

Please replace the paragraph beginning at page 3, line 25, with the following:

--**Figure 5** illustrates a comparison of Invader<sup>TM</sup> probe performance with different numbers of PPGs (SEQ ID NOS:5-7).--

Please replace the paragraph (**Table 1**) beginning at page 39, line 26, with the following:

--Table 1

**Sequences of Oligonucleotide Probes and Complementary Targets**

<u>Complementary Targets</u>	SEQ ID NO:	<u>Py/Pu-rich probes</u>	<u>base pairs</u>
1* TCGGCGGCGT	8	1*.MGB-Q-CGCCGCCG	8 G/C
2* ACAGCGGCGT	9	2* MGB-Q-CGCCGCTG	7 G/C, 1 A/T
3* ACAGCGACGT	10	3*.MGB-Q-CGTCGCTG	6 G/C, 2 A/T
4* TCAGTGACGA	11	4*.MGB-Q-CGTCACTG	5 G/C, 3 A/T
5* TCAGTGACAA	12	5*.MGB-Q-TGTCACTG	4 G/C, 4 A/T
6* TCAATGACAG	13	6*.MGB-Q-TGTCATTG	3 G/C, 5 A/T
7* ACAATGATAA	14	7*.MGB-Q-TATCATTG	2 G/C, 6 A/T
8* CCAATAATAA	15	8*.MGB-Q-TATTATTG	1 G/C, 7 A/T
9* GTAATAATAA	16	9*.MGB-Q-TATTATTA	8 A/T

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Please replace the paragraph (Table 3a and 3b) beginning at page 80, line 10, with the following:

--Table 3a and 3b  
Probe and target sequences

A. Probe Sequences – Mismatch Underlined			
Number	Mismatch	Probe Sequence	SEQ ID NO:
1	Complement	AAAGTTATGTCTACTTACAGAAA	17
2	A/C	AAAGCTATGTCTACTTACAGAAA	18
3	A/C	AAAGTCATGTCTACTTACAGAAA	19
4	T/G	AAAGTTGTGTCTACTTACAGAAA	20
5	A/C	AAAGTTACGTCTACTTACAGAAA	21
6	C/A	AAAGTTATATCTACTTACAGAAA	22
7	A/C	AAAGTTATGCCTACTTACAGAAA	23
8	G/T	AAAGTTATGTTTACTTACAGAAA	24
9	A/C	AAAGTTATGTCCACTTACAGAAA	25
10	T/G	AAAGTTATGTCTGCTTACAGAAA	26
11	G/T	AAAGTTATGTCTATTTACAGAAA	27
12	A/C	AAAGTTATGTCTACCTACAGAAA	28
13	A/C	AAAGTTATGTCTACTCACAGAAA	29
14	T/G	AAAGTTATGTCTACTTGCAGAAA	30
B. Target Sequences – A'=PPPA			
1	GTAAGTAGACATAAC		31
2	GTA'A'GTA'GA'CA'TA'A'C		32
3	GTAAGTAGACATAAC-MGB		33
4	GTA'A'GTA'GA'CA'TA'A'C-MGB		34

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Please replace the paragraph (Table 4) beginning at page 81, line 12, with the following:

--Table 4

**Comparison of thermodynamic discrimination of mismatched base pairs formed by  
HOPPPA or HOPU vs PPPA and PU in the 8-mer duplexes (+MGB).**

	Sequence of Duplex	SEQ ID NO:	PPPA/ PU	HOPPPA/ HOPU		Sequence of Duplex	SEQ ID NO:	PPPA/ PU	HOPPPA/ HOPU
			$\Delta\Delta G$ cal/mol	$\Delta\Delta G$ cal/mol				$\Delta\Delta G$ cal/mol	$\Delta\Delta G$ cal/mol
Match	CGUCACUG-MGB AGCTGTGACT	35			Match	UAUUAUUG-MGB AATAATAACC	45		
1	CGUCACUG-MGB AGCTGTGACT	36	4250	4350	10	UAUUAUUG-MGB AATTATAACC	46	4400	5000
2	CGUCACUG-MGB AGCGGTGACT	37	3450	3540	11	UAUUAUUG-MGB AATGATAACC	47	3740	3760
3	CGUCACUG-MGB AGCCGTGACT	38	4860	4530	12	UAUUAUUG-MGB AATCATAACC	48	6630	6840
4	CGUCACUG-MGB AGCAGAGACT	39	4870	4850	13	UAUUAUUG-MGB AATAAAAACC	49	5090	5730
5	CGUCACUG-MGB AGCAGGGACT	40	4190	4360	14	UAUUAUUG-MGB AATAAGAACC	50	5920	6520
6	CGUCACUG-MGB AGCAGCGACT	41	3930	3940	15	UAUUAUUG-MGB AATAACAACC	51	4120	4530
7	CGUCACUG-MGB AGCAATGACT	42	2600	2300					
8	CGUCACUG-MGB AGCATTGACT	43	4360	4210					
9	CGUCACUG-MGB AGCACTGACT	44	4420	4610					

$\Delta\Delta G$  was calculated at 37°C.--

Please replace the paragraph (Table 5, heading) beginning at page 83, line 17, with the following:

--Table 5

Comparison of <sup>32</sup>P-incorporation in primer extension product by polyacrylamide gel electrophoresis using AAC CAC TCT GTC CTA (SEQ ID NO:52) template--

Please replace the paragraph (Table 6) beginning at page 84, line 17, with the following:

--Table 6

Comparison of experimental  $T_m$ s with that of predicted  $T_m$ s using the nearest-neighbor thermodynamic parameters for PPG containing oligonucleotides and PPG containing oligonucleotides attached to a MGB

5'-Probe Sequence -3'	SEQ ID NO:	ODN Duplex Stability °C			MGB-ODN Duplex Stability °C		
		$T_{m_{exp}}$	$T_{m_{calc}}$	Err	$T_{m_{exp}}$	$T_{m_{calc}}$	Err
CTGTAAGTAGATATAAC	53	51.84	53.23	1.39	65.88	66.69	0.81
GGCAAGATATATAG	54	50.21	49.81	-0.40	66.37	65.56	-0.81
GTGACGCAGATTCC	55	61.27	61.06	-0.21	76.97	75.19	-1.78
GTAAGTAGACATAAC	56	52.12	51.78	-0.34	64.64	63.31	-1.33
CAGGGAGCTTTGGA	57	59.9	60.22	0.32	74.39	71.47	-2.92
CACTCGTGAAGCTG	58	60.85	59.49	-1.36	74.04	72.26	-1.78
GTAAGTAGGCATAAC	59	55.74	55.47	-0.27	66.91	66.00	-0.91
CCGGATGTAGGATC	60	57.52	59.05	1.53	69.3	70.03	0.73
GATTACCTGGATTT	61	50.64	50.32	-0.32	62.29	62.33	0.04
CCGTCAATGGTCAC	62	58.66	60.01	1.35	70.13	69.91	-0.22
CAGCACGTAGCC	63	57.31	58.07	0.76	69.29	67.60	-1.69
CGGCTACGTGCTGG	64	65.19	66.01	0.82	76.12	74.79	-1.33
CGGCTACATGCTGG	65	61.14	61.95	0.81	71.56	72.99	1.43
CTAAATCTGCCG	66	50.4	48.09	-2.31	62.08	60.19	-1.89
TCTGGATGATGGGCA	67	61.74	61.95	0.21	71.65	72.13	0.48
GTTCATGGGTGTAAT	68	57.51	57.77	0.26	66.94	68.79	1.85
CGGAGGTAGGATCA	69	59.24	59.46	0.22	69.46	70.93	1.47
CCACCCGCCTCAG	70	60.73	61.14	0.41	71.43	70.74	-0.69
CACAGGAGTGGTTGG	71	63.07	64.40	1.33	72.28	72.92	0.64
CGGACCAGTGCGTG	72	68.1	67.58	-0.52	77.92	76.80	-1.12
TCGGACCAGTGCGT	73	65.04	66.00	0.96	74.94	75.62	0.68
AACGGGGTACGATA	74	57.93	57.11	-0.82	67.79	67.08	-0.71
CAGTTGAGATTCTAAGAC	75	60.06	60.15	0.09	67.15	67.43	0.28
AGGGGCGTCTTG	76	60.78	58.57	-2.21	71.62	72.76	1.14
GTAAGTAGGCATAGC	77	58.34	58.95	0.61	65.95	66.99	1.04
TGCCCAGCCCCAG	78	63.13	63.40	0.27	71.28	71.32	0.04
CCAACACTCGTGAA	79	54.87	56.14	1.27	62.07	63.54	1.47
GTAAGTAGACACAGC	80	59.48	58.41	-1.07	65.79	66.27	0.48
TCGGACCAGTGC	81	58.02	58.55	0.53	65.99	66.35	0.36
CGATCACGCTGGC	82	62.12	62.75	0.63	69.18	71.81	2.63
GTCCTGGGGGTGG	83	65.19	64.54	-0.65	72.78	72.53	-0.25
GTAAGTAGGTGTGAC	84	60.7	59.70	-1.00	66.92	67.00	0.08

GGTTGTACGGGTTTCACG	85	<b>68.38</b>	68.81	0.43	<b>74.16</b>	75.38	1.22
GGACCAGTGCGTGA	86	<b>66.84</b>	65.46	-1.38	<b>73.38</b>	71.53	-1.85
GTAAGTAGACGCAGC	87	<b>62.91</b>	62.44	-0.47	<b>68</b>	67.82	-0.18
GTAAGTAGGCGCAGC	88	<b>65.52</b>	65.91	0.39	<b>69.8</b>	70.34	0.54
GTAAGTAGGCGCGGC	89	<b>68.71</b>	68.96	0.25	<b>72.26</b>	72.76	0.50
GGTTCCCGAGCG	90	<b>62.15</b>	61.14	-1.01	<b>65.75</b>	64.22	-1.53

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Please insert the accompanying paper copy of the Sequence Listing, page numbers 1 to 21, at the end of the application.

REMARKS

In accordance with 37 C.F.R. §§1.821 to 1.825, Applicants request entry of this amendment. This amendment is accompanied by a floppy disk containing SEQ ID NOS:1-90, in computer readable form, and a paper copy of the sequence information which has been printed from the floppy disk.

The information contained in the computer readable disk was prepared through the use of the software program "PatentIn" and is identical to that of the paper copy. This amendment contains no new matter.

Attached hereto is a marked-up version of the changes made to the Specification by the current Amendment. The attached pages are captioned "**VERSION WITH MARKINGS TO SHOW CHANGES MADE.**"

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PATENT

If the Examiner believes a telephone conference would expedite prosecution of this application, please telephone the undersigned at 415-576-0200.

Respectfully submitted,



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**VERSION WITH MARKINGS TO SHOW CHANGES MADE**

**In the Specification:**

Paragraph beginning at line 19 of page 3 has been amended as follows:

**Figure 3** is a chart which illustrates one advantage achieved through the use of PPPA and PPG in MGB-modified oligonucleotide probes. As seen in the figure, the modified bases allow shortening of the probe that shows increased mismatch discrimination in real-time PCR.  $\tilde{A}$  is PPPA and  $\tilde{G}$  is PPG. MGB-modified FAM probe = SEQ ID NO:1; MGB-modified FAM probe complement = SEQ ID NO:2; PPPA and PPG containing MGB-modified FAM probe complement = SEQ ID NO:3; PPPA and PPG containing MGB-modified FAM probe = SEQ ID NO:4.

Paragraph beginning at line 25 of page 3 has been amended as follows:

**Figure 5** illustrates a comparison of Invader<sup>TM</sup> probe performance with different numbers of PPGs (SEQ ID NOS:5-7).



Paragraph (**Table 1**) beginning at line 26 of page 39 has been amended as follows:

**Table 1**  
**Sequences of Oligonucleotide Probes and Complementary Targets**

<u>Complementary Targets</u>	<u>SEQ ID</u> <u>NO:</u>	<u>Py/Pu-rich probes</u>	<u>base</u> <u>pairs</u>
1* TCGGCGGCGT	<u>8</u>	1*.MGB-Q-CGCCGCCG	8 G/C
2* ACAGCGGCGT	<u>9</u>	2* MGB-Q-CGCCGCTG	7 G/C, 1 A/T
3* ACAGCGACGT	<u>10</u>	3*.MGB-Q-CGTCGCTG	6 G/C, 2 A/T
4* TCAGTGACGA	<u>11</u>	4*.MGB-Q-CGTCACTG	5 G/C, 3 A/T
5* TCAGTGACAA	<u>12</u>	5*.MGB-Q-TGTCACTG	4 G/C, 4 A/T
6* TCAATGACAG	<u>13</u>	6*.MGB-Q-TGTCATTG	3 G/C, 5 A/T
7* ACAATGATAA	<u>14</u>	7*.MGB-Q-TATCATTG	2 G/C, 6 A/T
8* CCAATAATAA	<u>15</u>	8*.MGB-Q-TATTATTG	1 G/C, 7 A/T
9* GTAATAATAA	<u>16</u>	9*.MGB-Q-TATTATTA	8 A/T

Paragraph (Table 3a and 3b) beginning at line 10 of page 80 has been amended as follows:

**Table 3a and 3b**  
**Probe and target sequences**

<b>A. Probe Sequences – Mismatch Underlined</b>			
<b>Number</b>	<b>Mismatch</b>	<b>Probe Sequence</b>	<b><u>SEQ ID NO:</u></b>
1	Complement	AAAGTTATGTCTACTTACAGAAA	<u>17</u>
2	A/C	AAAGCTATGTCTACTTACAGAAA	<u>18</u>
3	A/C	AAAGTCATGTCTACTTACAGAAA	<u>19</u>
4	T/G	AAAGTTGTGTCTACTTACAGAAA	<u>20</u>
5	A/C	AAAGTTACGTCTACTTACAGAAA	<u>21</u>
6	C/A	AAAGTTATATCTACTTACAGAAA	<u>22</u>
7	A/C	AAAGTTATGCCTACTTACAGAAA	<u>23</u>
8	G/T	AAAGTTATGTTTACTTACAGAAA	<u>24</u>
9	A/C	AAAGTTATGTCCACTTACAGAAA	<u>25</u>
10	T/G	AAAGTTATGTCTGCTTACAGAAA	<u>26</u>
11	G/T	AAAGTTATGTCTATTACAGAAA	<u>27</u>
12	A/C	AAAGTTATGTCTACCTACAGAAA	<u>28</u>
13	A/C	AAAGTTATGTCTACTCACAGAAA	<u>29</u>
14	T/G	AAAGTTATGTCTACTTGCAGAAA	<u>30</u>
<b>B. Target Sequences – A'=PPPA</b>			
1		GTAAGTAGACATAAC	<u>31</u>
2		GTA'A'GTA'GA'CA'TA'A'C	<u>32</u>
3		GTAAGTAGACATAAC-MGB	<u>33</u>
4		GTA'A'GTA'GA'CA'TA'A'C-MGB	<u>34</u>

Paragraph (Table 4) beginning at line 12 of page 81 has been amended as follows:

Table 4

Comparison of thermodynamic discrimination of mismatched base pairs formed by  
HOPPPA or HOPU vs PPPA and PU in the 8-mer duplexes (+MGB).

	Sequence of Duplex	<u>SEQ ID NO:</u>	PPPA/ PU	HOPPPA/ HOPU		Sequence of Duplex	<u>SEQ ID NO:</u>	PPPA/ PU	HOPPPA/ HOPU
			$\Delta\Delta G$ cal/mol	$\Delta\Delta G$ cal/mol				$\Delta\Delta G$ cal/mol	$\Delta\Delta G$ cal/mol
Match	CGUCACUG-MGB AGCTGTGACT	<u>35</u>			Match	UAUUAUUG-MGB AATAATAACC	<u>45</u>		
1	CGUCACUG-MGB AGCTGTGACT	<u>36</u>	4250	4350	10	UAUUAUUG-MGB AATTATAACC	<u>46</u>	4400	5000
2	CGUCACUG-MGB AGCGGTGACT	<u>37</u>	3450	3540	11	UAUUAUUG-MGB AATGATAACC	<u>47</u>	3740	3760
3	CGUCACUG-MGB AGCCGTGACT	<u>38</u>	4860	4530	12	UAUUAUUG-MGB AATCATAACC	<u>48</u>	6630	6840
4	CGUCACUG-MGB AGCAGAGACT	<u>39</u>	4870	4850	13	UAUUAUUG-MGB AATAAAACC	<u>49</u>	5090	5730
5	CGUCACUG-MGB AGCAGGGACT	<u>40</u>	4190	4360	14	UAUUAUUG-MGB AATAAGAACC	<u>50</u>	5920	6520
6	CGUCACUG-MGB AGCAGCGACT	<u>41</u>	3930	3940	15	UAUUAUUG-MGB AATAACAACC	<u>51</u>	4120	4530
7	CGUCACUG-MGB AGCAATGACT	<u>42</u>	2600	2300					
8	CGUCACUG-MGB AGCATTGACT	<u>43</u>	4360	4210					
9	CGUCACUG-MGB AGCACTGACT	<u>44</u>	4420	4610					

$\Delta\Delta G$  was calculated at 37°C.

Paragraph (Table 5, heading) beginning at line 18 of page 83 has been amended as follows:

Table 5

Comparison of <sup>32</sup>P-incorporation in primer extension product by polyacrylamide gel electrophoresis using AAC CAC TCT GTC CTA (SEQ ID NO:52) template

Paragraph (Table 6) beginning at line 17 of page 84 has been amended as follows:

**Table 6**

Comparison of experimental  $T_m$ s with that of predicted  $T_m$ s using the nearest-neighbor thermodynamic parameters for PPG containing oligonucleotides and PPG containing oligonucleotides attached to a MGB

5'-Probe Sequence -3'	SEQ ID NO:	ODN Duplex Stability °C			MGB-ODN Duplex Stability °C		
		$T_{m_{exp}}$	$T_{m_{calc}}$	Err	$T_{m_{exp}}$	$T_{m_{calc}}$	Err
CTGTAAGTAGATATAAC	<u>53</u>	<b>51.84</b>	53.23	1.39	<b>65.88</b>	66.69	0.81
GGCAAGATATATAG	<u>54</u>	<b>50.21</b>	49.81	-0.40	<b>66.37</b>	65.56	-0.81
GTGACGCAGATTCC	<u>55</u>	<b>61.27</b>	61.06	-0.21	<b>76.97</b>	75.19	-1.78
GTAAGTAGACATAAC	<u>56</u>	<b>52.12</b>	51.78	-0.34	<b>64.64</b>	63.31	-1.33
CAGGGAGCTTTGGA	<u>57</u>	<b>59.9</b>	60.22	0.32	<b>74.39</b>	71.47	-2.92
CACTCGTGAAGCTG	<u>58</u>	<b>60.85</b>	59.49	-1.36	<b>74.04</b>	72.26	-1.78
GTAAGTAGGCATAAC	<u>59</u>	<b>55.74</b>	55.47	-0.27	<b>66.91</b>	66.00	-0.91
CCGGATGTAGGATC	<u>60</u>	<b>57.52</b>	59.05	1.53	<b>69.3</b>	70.03	0.73
GATTACCTGGATTT	<u>61</u>	<b>50.64</b>	50.32	-0.32	<b>62.29</b>	62.33	0.04
CCGTCAATGGTCAC	<u>62</u>	<b>58.66</b>	60.01	1.35	<b>70.13</b>	69.91	-0.22
CAGCACGTAGCC	<u>63</u>	<b>57.31</b>	58.07	0.76	<b>69.29</b>	67.60	-1.69
CGGCTACGTGCTGG	<u>64</u>	<b>65.19</b>	66.01	0.82	<b>76.12</b>	74.79	-1.33
CGGCTACATGCTGG	<u>65</u>	<b>61.14</b>	61.95	0.81	<b>71.56</b>	72.99	1.43
CTAAATCTGCCG	<u>66</u>	<b>50.4</b>	48.09	-2.31	<b>62.08</b>	60.19	-1.89
TCTGGATGATGGGCA	<u>67</u>	<b>61.74</b>	61.95	0.21	<b>71.65</b>	72.13	0.48
GTTTCATGGGTGTAAT	<u>68</u>	<b>57.51</b>	57.77	0.26	<b>66.94</b>	68.79	1.85
CGGAGGTAGGATCA	<u>69</u>	<b>59.24</b>	59.46	0.22	<b>69.46</b>	70.93	1.47
CCACCCGCCTCAG	<u>70</u>	<b>60.73</b>	61.14	0.41	<b>71.43</b>	70.74	-0.69
CACAGGAGTGGTTGG	<u>71</u>	<b>63.07</b>	64.40	1.33	<b>72.28</b>	72.92	0.64
CGGACCAGTGCGTG	<u>72</u>	<b>68.1</b>	67.58	-0.52	<b>77.92</b>	76.80	-1.12
TCGGACCAGTGCGT	<u>73</u>	<b>65.04</b>	66.00	0.96	<b>74.94</b>	75.62	0.68
AACGGGGTACGATA	<u>74</u>	<b>57.93</b>	57.11	-0.82	<b>67.79</b>	67.08	-0.71
CAGTTGAGATTCTAAGAC	<u>75</u>	<b>60.06</b>	60.15	0.09	<b>67.15</b>	67.43	0.28
AGGGGCGTCTTG	<u>76</u>	<b>60.78</b>	58.57	-2.21	<b>71.62</b>	72.76	1.14
GTAAGTAGGCATAGC	<u>77</u>	<b>58.34</b>	58.95	0.61	<b>65.95</b>	66.99	1.04
TGCCCAGCCCCAG	<u>78</u>	<b>63.13</b>	63.40	0.27	<b>71.28</b>	71.32	0.04
CCAACACTCGTGAA	<u>79</u>	<b>54.87</b>	56.14	1.27	<b>62.07</b>	63.54	1.47
GTAAGTAGACACAGC	<u>80</u>	<b>59.48</b>	58.41	-1.07	<b>65.79</b>	66.27	0.48
TCGGACCAGTGC	<u>81</u>	<b>58.02</b>	58.55	0.53	<b>65.99</b>	66.35	0.36
CGATCACGCTGGC	<u>82</u>	<b>62.12</b>	62.75	0.63	<b>69.18</b>	71.81	2.63
GTCCTGGGGGTGG	<u>83</u>	<b>65.19</b>	64.54	-0.65	<b>72.78</b>	72.53	-0.25
GTAAGTAGGTGTGAC	<u>84</u>	<b>60.7</b>	59.70	-1.00	<b>66.92</b>	67.00	0.08

GGTTGTACGGGTTTCACG	<u>85</u>	<b>68.38</b>	68.81	0.43	<b>74.16</b>	75.38	1.22
GGACCAGTGCGTGA	<u>86</u>	<b>66.84</b>	65.46	-1.38	<b>73.38</b>	71.53	-1.85
GTAAGTAGACGCAGC	<u>87</u>	<b>62.91</b>	62.44	-0.47	<b>68</b>	67.82	-0.18
GTAAGTAGGCGCAGC	<u>88</u>	<b>65.52</b>	65.91	0.39	<b>69.8</b>	70.34	0.54
GTAAGTAGGCGCGGC	<u>89</u>	<b>68.71</b>	68.96	0.25	<b>72.26</b>	72.76	0.50
GGTTCCTCGAGCG	<u>90</u>	<b>62.15</b>	61.14	-1.01	<b>65.75</b>	64.22	-1.53